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Beneficial effect of donor-specific blood transfusions (DST) on living-related kidney allograft survival.

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Abstract

The survival rate of 19 patients who underwent living-related kidney transplantation after donor-specific blood transfusions (DST) was compared with that of 32 historical controls receiving transplants without DST. The graft survival rate of the DST group was 82% after two and three years. The graft survival rate of the DST group was significantly better than the 53% rate after two years obtained with the 32 historical controls (p less than 0.05). We tested sera from 16 DST-treated recipients to study the beneficial effect of DST on kidney allograft survival using the mixed lymphocyte culture (MLC) serum inhibition test. The results demonstrated that MLC inhibitory factors were induced in the serum of the recipient after completion of DST. This inhibition of MLC was observed by treatment of responder lymphocytes with serum obtained three weeks after DST plus rabbit complement. The inhibitory effect was also specific for responder cells in anti-donor MLC. Regarding the correlation with rejection episodes, these MLC inhibitory factors were often observed in the non-rejection group (p less than 0.05). The data suggest that such factors may be anti-idiotypic antibodies and be associated with prolonged graft survival.

KEYWORDS: kidney transplantation, donor-specific blood transfusion (DST), MLC inhibitory factors, anti-idiotypic antibody.

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Beneficial Effect of Donor-Specific Blood Transfusions (DST) on Living-Related Kidney Allograft Survival

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The survival rate of 19 patients who underwent living-related kidney transplantation after donor-specific blood transfusions (DST) was compared with that of 32 historical controls receiving transplants without DST. The graft survival rate of the DST group was 82% after two and three years. The graft survival rate of the DST group was significantly better than the 53% rate after two years obtained with the 32 historical controls ($p < 0.05$). We tested sera from 16 DST-treated recipients to study the beneficial effect of DST on kidney allograft survival using the mixed lymphocyte culture (MLC) serum inhibition test. The results demonstrated that MLC inhibitory factors were induced in the serum of the recipient after completion of DST. This inhibition of MLC was observed by treatment of responder lymphocytes with serum obtained three weeks after DST plus rabbit complement. The inhibitory effect was also specific for responder cells in anti-donor MLC. Regarding the correlation with rejection episodes, these MLC inhibitory factors were often observed in the non-rejection group ($p < 0.05$). The data suggest that such factors may be anti-idiotypic antibodies and be associated with prolonged graft survival.

Key words : kidney transplantation, donor-specific blood transfusion (DST), MLC inhibitory factors, anti-idiotypic antibody.

Pretransplant blood transfusions were originally reported by Opelz *et al.* (1) as improving cadaveric renal allograft survival rates. Numerous subsequent reports have confirmed this report. A protocol for deliberate pretransplant donor-specific blood transfusions (DST) was reported by San-Francisco groups (2) in 1980. DST provided to one-haplotype-mismatched, renal graft recipients have resulted in significant improvement in allograft survival rates (3-5). Our experience with the outcome and immune modulation of patients receiving a living-related one-haplotype-mismatched kidney from their blood donor is analysed in this report.

Materials and Methods

Study population. One hundred and seven kidney transplantations were performed between March, 1974 and April, 1985 (Fig. 1). Since May, 1982, 22 prospective kidney transplant recipients received DST from a living-related one-haplotype-mismatched donor. Twenty patients received DST from their parents and one from a sibling. Nineteen patients who remained negative in direct lymphocyte crossmatch after DST received a kidney from their blood donor within three months of completion of DST.

The survival rate of the 19 patients who underwent kidney transplantation after DST was compared with that of 32 historical controls who received transplants without DST. Patient charac-

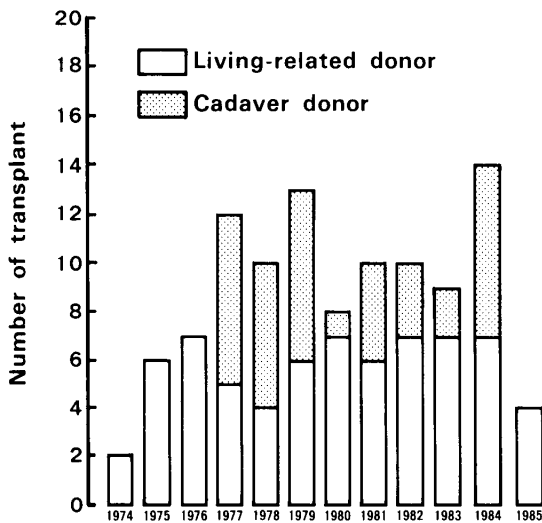


Fig. 1 Number of kidney transplants by year at the University of Okayama between March, 1974 and April, 1985.

teristics for both groups are compared in Table 1. There were no significant differences in pretransplant risk factors of either group, and the recipients were treated similarly with methylprednisolone and azathioprine.

DST protocol. Three transfusions each of approximately 200 ml fresh whole blood from the potential kidney donor were given at two-week intervals. Serum samples and peripheral blood lymphocytes (PBLs) obtained before DST (pre-DST) and three weeks after the last transfusion

(post-DST) were cryo-preserved prior to their use in a mixed lymphocyte culture (MLC) serum inhibition test.

MLC serum inhibition test. This test was designed to assess the effect of recipient's serum on MLC as described elsewhere (6). Our purpose was to determine whether the results of this test correlate with the rejection episode. In brief, responder cells (5×10^6) were treated with 1 : 2 diluted serum obtained pre-DST or post-DST at 4°C for 30 minutes, washed twice and incubated with a 1 : 8 dilution of rabbit complement at 37°C for 60 minutes. After washing twice, responder cells were prepared at a concentration of 5×10^4 cells/0.1 ml. Stimulator cells treated with mitomycin-C were also suspended at 5×10^4 cells/0.1 ml. The cultures were prepared in quadruplicate and incubated in round-bottomed 96-well microtiter plates (Corning, N.Y.) in a 95% air-5% CO₂ atmosphere for 144 h. After 120 h. of culturing, 1 μ Ci/well [³H]-thymidine was added. At the end of the incubation period, the cultures were harvested with a multiple automatic sample harvester. [³H]-thymidine incorporated by the PBLs was determined in a liquid scintillation counter. The results were expressed as the percentage of MLC inhibition according to the following formula:

% MLC inhibition

$$= \left(1 - \frac{\text{mean CPM in R}^* \text{ Dm}}{\text{mean CPM in R}^0 \text{ Dm}} \right) \times 100,$$

where R⁰ Dm means a MLC combination in which

Table 1 Patient characteristics^a

| | DST group | Non-DST group |
|---|-----------------------------|-----------------|
| Number of patients | 19 | 32 |
| Male/female | 13/6 | 20/12 |
| Mean patient age (years) | 29.1 \pm 6.1 ^b | 26.1 \pm 4.8 |
| Mean dialysis period (months) | 28.9 \pm 11.2 | 29.7 \pm 20.1 |
| Mean number of pretransplant third-party blood transfusions (units) | 11.2 \pm 7.8 | 14.5 \pm 13.2 |
| HLA-A,B matching | Haploidentical | Haploidentical |
| Mean relative response of MLC ^c (%) | 78.2 \pm 58.2 | 68.9 \pm 53.2 |
| Immunosuppressants (one-month) | | |
| Methylprednisolone (mg) | 1601 \pm 185 | 1752 \pm 83 |
| Azathioprine (mg) | 3740 \pm 509 | 3687 \pm 287 |

^a : Significant differences were not found between the groups

^b : mean \pm SD, ^c : Mixed Lymphocyte Culture

the responder cells (R) were pretreated with pre-DST serum plus complement, and R° Dm means a MLC combination in which the responder cells were pretreated with post-DST serum plus complement.

Results

Graft survival. Of the 22 patients who entered the DST protocol, 90% received transplants and 10% became sensitized. Results of transplantation in the DST group were better than in the non-DST groups, as shown in Fig. 2. The actuarial percentage

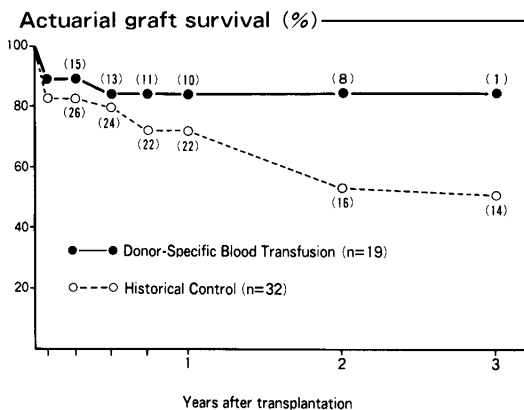


Fig. 2 Graft survival rates of 19 patients who received DST (●) and 32 historical control patients (○) who did not receive DST.

of patients alive and with functioning kidneys one year after transplantation was 82% in the DST group, and 73% in the non-DST historical control group. The graft survival rate of the DST group was 82% after two and three years. Thus, the graft survival rate was significantly better than the 53% rate after two years obtained with the 32 historical controls who received transplants without DST ($p < 0.05$).

A kinetics study of the MLC serum inhibition test following each DST. This study was performed on six patients who received three DSTs. As shown in Fig. 3, anti-donor

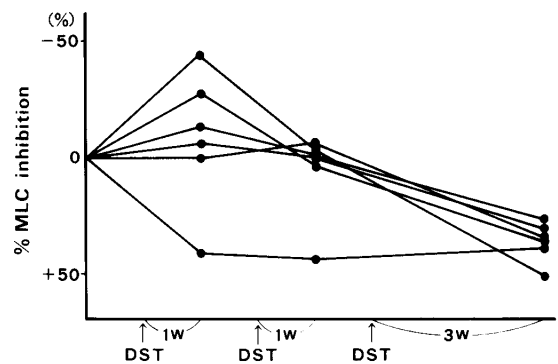


Fig. 3 Kinetics study of the MLC serum inhibition test following each DST.

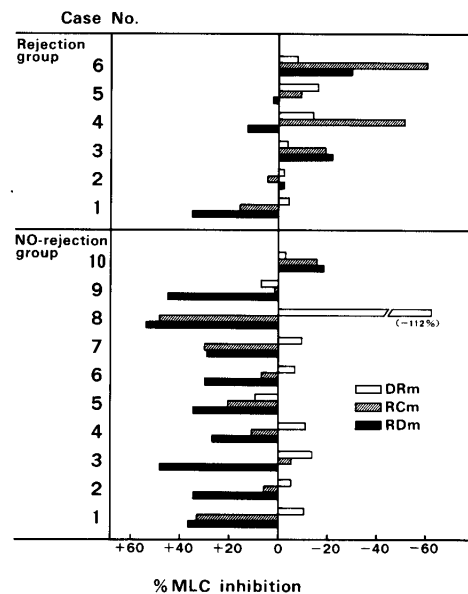


Fig. 4 Correlation between the results of the MLC serum inhibition test and rejection episodes. Three kinds of MLC combination (RDm, RCm, DRm, R = recipient lymphocytes; D = donor lymphocytes; C = third party lymphocytes; m = mitomycin C treated) were set up and the results were expressed as the percentage inhibition of MLC by the pretreatment of responder cells with post-DST serum plus complement.

MLC responses using responder cells pretreated with the serum plus complement one week after the first or second DST were slightly augmented or similar to those of cells pretreated with pre-DST serum. By contrast, responses 3 weeks after the third

DST were markedly inhibited.

MLC serum inhibition test and rejection episodes. This assay was performed on 16 patients who received three DSTs. In six out of the 16 patients, rejection occurred. The results of the MLC serum inhibition test were compared with the presence or absence of rejection episodes (Fig. 4). The percentage inhibition of anti-donor MLC (RDm) by the pretreatment of responder cells with post-DST serum plus complement was from 27.5% to 59.0% in nine out of the ten patients in the non-rejection group. By contrast, pronounced inhibition occurred in only one case out of the six patients in the rejection group ($p < 0.05$).

In addition, these MLC inhibitory sera showed no suppressive activity in MLC combinations of DRm in any of the cases, and less than 20% inhibition in MLC response with third party cells (RCm) in eight out of eleven cases. The above results indicate that the MLC inhibitory factors which are induced in the serum of potential kidney transplant recipients after the completion of DST are specifically directed against the recipients' own PBLs, and not blood donor PLBs.

Discussion

The present study showed that donor-specific blood transfusions, as pretransplant conditioning, result in improved kidney graft survival in living-related one-haplotype-mismatched donor-recipient combinations. Similar results have been reported by others (2-5). Historically, kidney allografts from one-haplotype-mismatched living donors in patients not given DST were frequently rejected within the first year after transplant, with 60-65% having a 1-year graft survival (3, 7).

The beneficial effect of pretransplant donor-specific transfusions on the survival of

human kidney allografts is well established (2-5), but the mechanisms involved in the DST effect remain a source of controversy. Several hypotheses have been proposed to explain this effect, such as recipient selection, decloning and autoregulation by suppressor cells or antiidiotypic antibodies.

Preliminary results suggesting a possible mechanism of the DST effect have been reported elsewhere (6). In the present study, we focused on the humoral factors that might be relevant to the beneficial effect of DST. Three striking findings have emerged from the present study: (1) the patients' anti-donor MLC response was specifically inhibited by the patients' sera taken three weeks following the completion of DST in 10 out of 16 DST-treated patients; (2) pretreatment of donor cells with the inhibitory patient sera did not reduce the anti-donor MLC response, whereas pretreatment of responder cells caused specific inhibition, and (3) induction of MLC inhibitory factors significantly ($p < 0.05$) reduced the incidence of rejection episodes.

A recent study in mice revealed that serum obtained 1-3 weeks after the last of 3 weekly transfusions from a blood donor with a different H-2 inhibited the MLC response to the blood donor strain but not to third-party stimulator cells (8). Such experimental data correlate well with our clinical findings.

Our present data indicate that post-DST serum and complement treatment of autologous DST-primed cells (recipients' PBLs after the completion of DST) blocked the ability of these cells to respond in MLC to the stimulator cells from the blood donor, but not to cells from third party donors. Our data also indicate that such post-DST sera might include antibodies directed against recognition sites on T lymphocytes, *i.e.*, anti-idiotypic antibodies. In conclusion, our studies have demonstrated that such anti-

idiotypic antibodies may be induced by DST and be involved in the mechanism producing a prolonging effect on graft survival.

References

1. Opelz G, Sengar D P S, Mickey M R and Terasaki P I: Effect of blood transfusions on subsequent kidney transplants. *Transplant Proc* (1973) **4**, 253-256.
2. Salvatierra O Jr, Vincenti F, Amend W, Potler D and Feduska N J: Deliberate donor-specific blood transfusions prior to living related renal transplantation. *Ann Surg* (1980) **192**, 543-552.
3. Yamauchi J, Yamada Y, Otsubo O and Inou T: Prolongation of kidney transplant survival by donor-specific blood transfusion. *Transplant Proc* (1983) **15**, 932-935.
4. Glass N R, Miller D T, Sollinger H W and Belzer F O: A four-year experience with donor blood transfusion protocols for living-donor renal transplantation. *Transplantation* (1985) **39**, 615-619.
5. Cheigh J S, Suthanthiran M, Kaplan M, Stubenbord W T, Stenzel K H and Rubin A L: Induction of immune alternations and successful renal transplantation with a simplified method of donor-specific blood transfusion. *Transplantation* (1985) **38**, 501-506.
6. Takeuchi H, Sakagami K, Seki Y, Tsuboi M, Matsumoto M, Miyazaki M and Orita K: Antiidiotypic antibodies and suppressor cells induced by donor-specific transfusion in potential kidney transplant recipients. *Transplant Proc* (1985) **17**, 1059-1061.
7. Krakour H: The kidney transplant histocompatibility study; in *Analysis of the Data, Phase One, an Overview*, National Institutes of Health ed, Vol 1, DHEW Publication Co, Washington DC (1980) pp 80-2164.
8. Nagarkatti P S, Joseph S and Single D P: Induction of antibodies by blood transfusions capable of inhibiting responses in MLC. *Transplantation* (1983) **36**, 695-699.

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